Caspase-1 Regulates the Inflammatory Process Leading to Autoimmune Demyelination[1]

Roberto Furlan,* Gianvito Martino,[2]*† Francesca Galbiati,‡ Pietro L. Poliani,* Simona Smiroldo,‡ Alessendra Bergami,* Gaetano Desina,*§ Giancarlo Comi,† Richard Flavell,¶ Michael S. Su,‖ and Luciano Adorini‡

T cell-mediated inflammation is considered to play a key role in the pathogenic mechanisms sustaining multiple sclerosis (MS). Caspase-1, formerly designated IL-1β-converting enzyme, is crucially involved in immune-mediated inflammation because of its pivotal role in regulating the cellular export of IL-1β and IL-18. We studied the role of caspase-1 in experimental autoimmune encephalomyelitis (EAE), the animal model for MS. Caspase-1 is transcriptionally induced during EAE, and its levels correlate with the clinical course and transcription rate of proinflammatory cytokines such as TNF-α, IL-1β, IFN-γ, and IL-6. A reduction of EAE incidence and severity is observed in caspase-1-deficient mice, depending on the immunogenicity and on the amount of the encephalitogenic myelin oligodendrocyte glycoprotein (MOG) peptide used. In caspase-1-deficient mice, reduced EAE incidence correlates with defective development of anti-MOG IFN-γ-producing Th1 cells. Finally, pharmacological blockade of caspase-1 in Biozzi AB/H mice, immunized with spinal cord homogenate or MOG_{35-55} peptide, by the caspase-1-inhibitor Z-Val-Ala-DL-Asp-fluoromethylketone, significantly reduces EAE incidence in a preventive but not in a therapeutic protocol. These results indicate that caspase-1 plays an important role in the early stage of the immune-mediated inflammatory process leading to EAE, thus representing a possible therapeutic target in the acute phase of relapsing remitting MS. *The Journal of Immunology,* **1999, 163: 2403–2409.**

The caspase family comprises thus far 13 different cysteine proteases that are mainly involved in the apoptotic pathway (1). Among them, caspase-1, formerly named IL-1β-converting enzyme, which is activated by caspase-11-mediated proteolytic cleavage (2), is less involved in the apoptotic cascade but is prominent in inflammation because of its pivotal role in regulating the cellular export of proinflammatory cytokines such as IL-1β. Caspase-1 is elevated in intestinal macrophages during inflammatory bowel disease (3) and in a variety of organs, including the brain, in response to bacterial LPS administration (4). Further evidence on the role played by caspase-1 in inflammation comes from studies on caspase-1-deficient (−/−) mice and caspase-1 pharmacological inhibitors. Caspase-$1^{-/-}$ mice display an alteration in the export of several proinflammatory cytokines, namely IL-1β, IL-1α, IL-6, and TNF-α, although neither IL-1α nor IL-6 nor TNF-α are substrates for caspase-1 (5). Furthermore, caspase-1 proteolytically activates IL-18, and caspase-$1^{-/-}$ mice have also reduced serum levels of IL-18 and IFN-γ in response to LPS administration (6). Caspase-$1^{-/-}$ mice are resistant to LPS-induced endotoxic shock (7) and to the induction of experimental pancreatitis (8). In vivo pharmacological inhibition of caspase-1 protects mice from TNF-α-induced liver failure (9) and collagen-induced arthritis (10).

MS[3] is an immune-mediated demyelinating disease of the CNS of unknown etiology (11). The pathological hallmark of the disease is the presence within the CNS of inflammatory infiltrates containing few autoreactive T cells and many pathogenic nonspecific mononuclear cells (12). It is currently believed that Ag-specific T cells provide the organ specificity of the pathogenic process and regulate the recirculation within the CNS of activated mononuclear cells releasing inflammatory myelinotoxic substances. These latter cells can be activated in the periphery by polyclonal inflammatory stimuli, thus determining disease recurrence (12, 13). Proinflammatory cytokines participate either in Ag-specific T cell activation or in peripheral activation of nonspecific mononuclear cells. TNF-α, IFN-γ, and IL-6 levels increase before disease relapses (13, 14). An increased number of disease relapses was observed in MS patients treated with IFN-γ (15). Moreover, TNF-α, IFN-γ, and IL-1β are present in demyelinating plaques (16), and IL-1β has been shown to be a mediator of the inflammatory process sustaining EAE, the animal model for MS (17).

We evaluated the role of caspase-1 in EAE. We found that caspase-1 mRNA blood levels parallel those of proinflammatory cytokines, such as IL-1β, IL-6, TNF-α, and IFN-γ, during EAE and peak at the time of maximal EAE severity. A reduction of EAE incidence and severity was observed in caspase-$1^{-/-}$ mice depending on the immunogenicity and on the amount of the encephalitogenic MOG peptide used. Finally, pharmacological blockade of caspase-1 reduced the incidence of EAE, induced either with SCH or MOG_{35-55} peptide, in a preventive but not therapeutic

*Experimental Neuroimmunotherapy Unit, Department of Biotechnology, and †Department of Neurology, San Raffaele Scientific Institute, Milan, Italy; ‡Roche Milano Ricerche, Milan, Italy; §Department of Neurology, Casa Sollievo della Sofferenza Scientific Institute, San Giovanni Rotondo (FG), Italy; ¶Howard Huges Medical Institute, Yale University School of Medicine, New Haven, CT 06510; and ‖Vertex Pharmaceuticals, Inc., Cambridge, MA 02139

Received for publication April 12, 1999. Accepted for publication June 10, 1999.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] This work was supported by Istituto Superiore di Sanità (Target project: Multiple Sclerosis), and Ministero dell'Università e della Ricerca Scientifica e Tecnologica.

[2] Address correspondence and reprint requests to Dr. Gianvito Martino, Department of Biotechnology-San Raffaele Scientific Institute, Via Olgettina 58, 20132 Milan, Italy. E-mail address: g.martino@hsr.it

[3] Abbreviations used in this paper: MS, multiple sclerosis; EAE, experimental autoimmune encephalomyelitis; MOG, myelin oligodendrocyte glycoprotein; SCH, spinal cord homogenate; AU, arbitrary units; p.i., postimmunization.

0022-1767/99/$02.00